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BIOLOGICAL OBSERVATIONS ON *AEDES SEATOI* HUANG IN THAILAND WITH NOTES ON RURAL *AEDES AEGYPTI* (L.) AND OTHER *STEGOMYIA* POPULATIONS

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Abstract: *Aedes seatoi* is recorded from 8 Thailand provinces. Observations show *A. seatoi* bites man, and suggest it is semi-domestic and usually selects natural bamboo containers for oviposition, rather than artificial containers. *Aedes albopictus*, *A. seatoi*, *A. annandalei*, *A. aegypti*, *A. gardnerii imitator* and *A. pseudalbopictus* were collected in rural villages by bamboo cup surveys in that order of frequency. Incidence of *A. aegypti* utilization of natural containers in rural villages was higher than anticipated and deserves serious consideration in future surveys. Larval associations of *Stegomyia* species in natural and artificial containers in rural village habitats were complex and add an additional taxonomic burden to surveys. This burden is due to the nearly identical larvae of *A. aegypti* and *A. seatoi* and nearly identical adults of *A. albopictus* and *A. seatoi*.

From 1966 to mid-1970 the Department of Medical Entomology, SEATO Medical Research Laboratory, Bangkok, was involved in several aspects of *Aedes* research in Thailand. Primary focus was research on *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse), and their vector status, ecology and control during dengue-hemorrhagic fever epidemics (Gould et al. 1968, 1970). Taxonomic investigations were coordinated with the Southeast Asia Mosquito Project (SEAMP), Smithsonian Institution. Basic information such as biting behavior, associated species, variety of habitats utilized and distribution were used to help define the species. Thousands of specimens of *Aedes* (*Stegomyia*) collected during that time are being studied by Dr Yiau-Min Huang of SEAMP.

An early benefit of this combined taxonomic-biologic study was the recognition of *Aedes* (*S.*) *scutellaris malayensis* Colless, previously unreported from Thailand (SEATO Med. Res. Lab., 1966, unpubl.). Further, in 1969 Huang described a new species (*Aedes* (*S.*) *seatoi*) with the fourth instar larvae almost identical to those of *A. aegypti* and the adults nearly identical with *A. albopictus*. The current study focused on learning more about the ecology, ethology and distribution of *A. seatoi*, and to attempt colonization. Additional data are also presented on natural container utilization by *A. aegypti*, and *Stegomyia* larval associations in natural habitats. These data emphasize the need (Eldridge 1969, Paterson 1970) for a firm, precise taxonomic base before biological field research is undertaken.

Limited biological data published with the original description of *A. seatoi* and unpublished records suggested *A. seatoi* normally uses natural containers for oviposition, while *A. aegypti*, outside of Africa, is renowned for its use of artificial containers. Since this basic biological difference seemed apparent, it was thought that old Thailand records of larval *A. aegypti* in natural containers might refer to *A. seatoi*. The reverse situation, *A. seatoi* in artificial containers, was also considered.

Previous published Thailand records of natural container breeding by *A. aegypti* (Scanlon 1965) refer to an earlier record (SEATO Med. Res. Lab., 1963, unpubl.) of *A. aegypti* larvae in tree holes in Bangkok. Studies on Samui Island in southern Thailand (Gould et al. 1968) revealed a low incidence of *A. aegypti* larvae in tree holes, coconut shells, husks and bracts. Yasuno & Tonn (1969) and Tonn et al. (1969, 1970) reported minimal *A. aegypti* larval utilization of plant axils, tree holes and coconut shells in the Bangkok area.

Other Oriental records of natural container use by *A. aegypti* larvae are as follows: Macdonald (1956), who reported *A. aegypti* in tree holes, tree stumps and bamboo stumps in Malaya; Gilotra et al. (1967), who collected *A. aegypti* in bamboo stumps and coconut shells in Calcutta, India; and Rao et al. (1970), who reported relatively frequent usage of tree holes by *A. aegypti* in towns in southern India.

The above reports emphasize the close association of these natural container sites to man. Feral strains of *A. aegypti* similar to those reported from

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South Africa (Muspratt 1956) have not been observed in Asia.

MATERIAL AND METHODS

Unpublished data gathered by entomology personnel of SEATO Laboratory during 1966–1970 indicated *A. seatoi* was readily attracted to bamboo cups for oviposition. Consequently, bamboo cup surveys were chosen as the primary larval collection method and only limited surveys were made of natural and in situ artificial containers. Some additional behavioral data was obtained by a few adult biting collections from humans.

Dried numbered bamboo cups (3–8 cm inside diameter and 20–30 cm deep) were placed in varied locations and filled with mosquito-free water. The number of cups set out varied with size of the survey area. Cups were left in the field for 7–14 days and checked at least once for larvae during that time. If larvae were found they were collected for rearing and the cups were refilled. At the end of the field period the cups were emptied into numbered plastic bags and both were taken to the laboratory, where the cups were refilled with water to hatch any eggs present and the plastic bags were emptied into enamel pans. These flooded cups were covered with a paper towel held in place by a rubber band to prevent extraneous oviposition, and were checked daily for larvae for another 7–10 days. All larvae collected from a given cup during the entire period were reared in 1 enamel pan and totaled under 1 collection. If the bamboo cups were needed for a second survey, they were cleaned by a combination of a steel brush, sand paper and boiling water.

Identifications for immature collections were based on reared adults, many reared individually with associated immature skins. Large numbers of *A. seatoi* adults were placed in cages for colonization.

Description of habitats

Two ecological habitats are described below. Although transitory situations are found, the 2 basic habitats are best defined in terms of the abundance of bamboo and village size.

(1) *Small Village-Bamboo Habitat.* Throughout the rice growing lowlands and valleys of Thailand are many small villages (5–30 houses). Almost invariably the villagers have planted thorny bamboos (*Bambusa* spp.) and large stem bamboos (*Dendrocalamus* spp.) around and/or throughout the village. These bamboo serve as a wind break, a protective wall against intruders, provide shade and are selectively harvested for making numerous items. Various fruit trees may be present, particularly in larger villages; however, the bamboos are the most abundant vegetation and provide the majority of natural containers.

(2) *Village-Orchard Habitat.* This is a common habitat in the valleys and coastal areas, usually associated with a major water source such as a river and larger, widespread villages. The trees are planted in mixed groves and include such fruits as mango (*Mangifera indica* Linn.), jackfruit (*Artocarpus integrifolia* Linn.), durien (*Durio zibethinus* Murr.), rambutan (*Nephelium lappaceum* Linn.), mangosteen (*Garcinia mangostana* Linn.) and other common fruits like banana, coconut, papaya and pineapple. Houses are normally near, and infrequently, located in the groves. Artificially introduced clones of bamboo are common, but tree holes, stump holes and plant axils provide the majority of natural containers.

RESULTS

Aedes seatoi

Data presented for *A. seatoi* in the original description listed collections in Chon Buri and Kanchanaburi Provinces from bamboo pots (=cups) in an orchard, village and a mangrove forest, and 2 collections from banana axils. Associated species were *A. aegypti*, *A. albopictus* and *Armigeres* sp. (Huang 1969).

Aedes seatoi distribution is herein extended by 6 provinces: Ang Thong, Chiang Mai, Lop Buri, Nakhon Sawan, Prachin Buri and Sara Buri (FIG. 1). These new records are from larval collections near human habitation, except the Prachin Buri records which stem from human biting collections.

The list of associated species is herein revised to include *A. aegypti*, *A. albopictus*, *A. annandalei* (Theobald), *A. gardnerii imitator* Leicester, *Armigeres magnus* (Theobald), *Ar. subalbatus* (Coquillett), *Culex brevipalpis* (Giles) and *Tripteroides aranoides* (Theobald.)

Biting records for *A. seatoi* consist of collections from humans from 3 villages in Sara Buri Province and the above records from Prachin Buri Province. Only 13 specimens have been collected biting, one between 1000–1100 hr, while the remaining specimens were collected between 1600–2100 hr. Nothing is known about the feeding response of *A. seatoi* to animals other than humans.

Natural container collections of *A. seatoi* larvae were limited to: bamboo stumps (1–3 m high) in 1 village in Lop Buri and 2 villages in Sara Buri

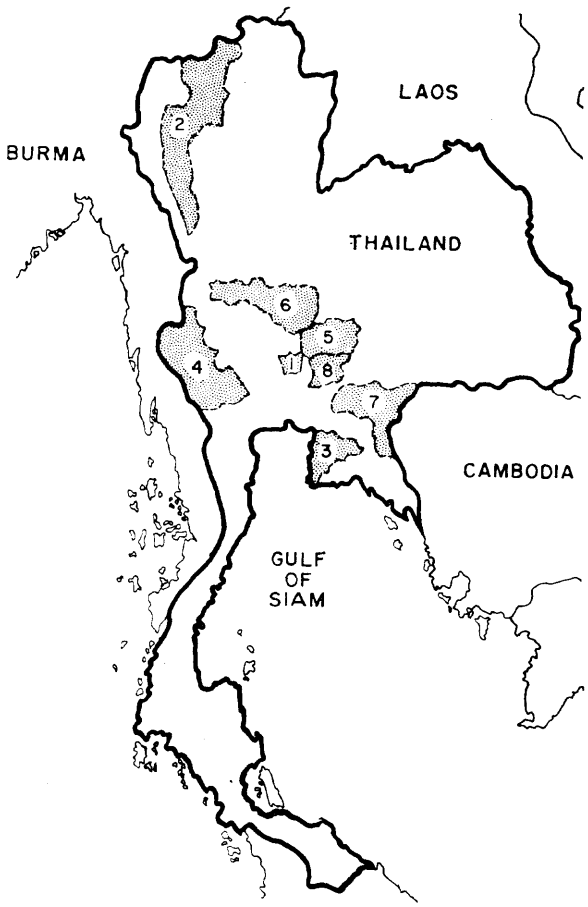

FIG. 1. Distribution of *Aedes seatoi* in Thailand. Provinces of Thailand: 1. Ang Thong. 2. Chiang Mai. 3. Chon Buri. 4. Kanchanaburi. 5. Lop Buri. 6. Nakhon Sawan. 7. Prachin Buri. 8. Sara Buri.

Provinces; the stump of a mango tree in Sara Buri Province; and a split bamboo collection in Nakhon Sawan Province. Banana axils checked during the surveys were negative for *A. seatoi*. Unfortunately, most of the surveys were conducted during the hot-dry season and very few axils contained water.

Artificial container collections, other than bamboo cups, were made in 3 provinces. Of 99 outside water jars (ong) checked, only 1 jar contained *A. seatoi* larvae. This jar was located about 5 m from a house in Ang Thong Province.

Aedes seatoi was invariably the second most prevalent *Stegomyia* species in the bamboo cup surveys (TABLE 1). Only *A. albopictus* had a higher incidence (93%) as compared to *A. seatoi* (39%), while *A. aegypti* and *A. annandalei* (both 22%), *A. gardnerii imitator* (7%) and *A. pseudalbopictus* (0.5%) followed in that order. A small (28-cup) survey in drought-stricken Ang Thong Province was omitted from TABLE 1. Of those, only 2 cups set near a trail in dry deciduous scrub were positive, and both contained *A. albopictus* and *A. seatoi*.

Aedes aegypti

Aedes aegypti is widely distributed in Thailand in large communities along the major transportation routes (Scanlon 1966, Yasuno et al. 1969). Now, data suggest that this species is also widely distributed in the rural villages of the central valley.

An isolated population of *A. aegypti* was found in a deserted, mountain top community in Nakhon Sawan Province. This community had been completely abandoned for over 1 year and the nearest humans resided approximately 1.5 km away. Larvae of *A. aegypti*, *A. albopictus* and *A. pseudalbopictus* (Borel), were found in a large water jar and adult *A. aegypti* were collected biting man. This situation recalls one reported by Pearson & Harrison (1967), where an *A. aegypti* population was found on the deserted part of a military post in the U.S. (Alabama). In that situation the nearest humans were located 1071 m from the collection site and blooded *A. aegypti* females were found to have fed on cattle.

Two biting collections of *A. aegypti* were made more than 100 m from houses. The first involved *A. aegypti*, *A. albopictus*, *A. annandalei* and *A. gardnerii imitator* biting humans between 0900–1100 in a village-orchard habitat in Sara Buri Province. The second involved *A. aegypti*, *A. albopictus* and *Armigeres magnus* biting humans between 1600–1800 in a village-bamboo habitat in Lop Buri Province.

Natural container collections of *A. aegypti* larvae were recorded from Lop Buri, Nakhon Sawan, Phra Nakhon (Bangkok) and Sara Buri Provinces. Associated species from these collections were: *A. albopictus*, *A. annandalei*, *A. gardnerii imitator*, *A. seatoi*, *Armigeres subalbatus*, *Culex brevipalpis*, *Toxorhynchites* sp. and *Tripteroides aranoides*. The Nakhon Sawan record refers to a bamboo stump collection of *A. aegypti* in a small village south of the provincial capital. In Lop Buri Province, 3 of 11 bamboo stumps found with larvae contained *A. aegypti*. These stumps (1–3 m high) were 30, 50 and 100 m from houses in a village-bamboo habitat. Three of 15 bamboo stumps found with larvae in Sara Buri Province contained *A. aegypti*. These stumps (1–3 m high) were in a village-bamboo habitat and all within 15 m of the nearest house. Another collection of *A. aegypti* was made from the stump of a mango tree approximately 50 m from the nearest house, in a village-orchard habitat in Sara Buri Province. Four natural container

TABLE 1. Oviposition utilization of bamboo cups by *Stegomyia* spp.

Locality data	Total Cups	Positive for *STEGOMYIA*		Species incidence in positive *STEGOMYIA* cups											
				albopictus		*seatoi*		*annandalei*		*aegypti*		*gardnerii imitator*		*pseud-albo-pictus*	
		#	%	#	%	#	%	#	%	#	%	#	%	#	%
Ang Thong Village-orchard															
50–150 m*	199	81	41	78	96	18	22	–	–	6	7	3	4	1	1
Sara Buri Village-orchard															
20–50 m	41	26	63	25	96	10	39	6	23	6	23	5	19	–	–
Lop Buri Village-bamboo															
20–50 m	30	13	43	11	85	7	54	4	31	2	15	1	8	–	–
Sara Buri Village-bamboo															
10–15 m.	106	78	74	69	89	37	47	33	42	28	36	–	–	–	–
Sara Buri Hill Top Area															
300–400 m	20	18	90	18	100	13	72	5	28	5	28	6	33	–	–
Total	396	216	55	201	93	85	39	48	22	47	22	15	7	1	0.5

*Distance cups set from nearest houses.

collections of *A. aegypti* larvae were made on 2 premises in a residential area of Bangkok (Phra Nakhon Province). A search of the premises revealed no artificial container breeding, only the above sites plus 3 potential sites that were dry. The 4 collections involved (1) larvae found in water collected in the tunnel of a Palm Weevil (*Rhynchophorus* sp.) in the trunk of a coconut palm, (2) two collections made in water-retaining crotches of 2 flame trees [*Delonix regia* (Bojer) Raf. (Syn.= *Poinciana*)], and (3) larvae found in a receptacle formed by root flanges at the base of 1 of the flame trees.

Collections for *A. aegypti* and *A. seatoi* in clay or concrete water jars were made in villages in Ang Thong, Nakhon Sawan and Sara Buri Provinces. Of 99 outside jars checked, 36 were found negative for *Stegomyia*, 60 contained *A. aegypti*, 16 contained *A. albopictus*, 1 contained *A. pseudalbopictus* and significantly, only 1 contained *A. seatoi*. The *A. albopictus* and *A. pseudalbopictus* larvae were found mixed with *A. aegypti* except for 2 jars with *A. albopictus* alone, while the *A. seatoi* larvae were found alone. Other containers such as tin cans, bottles and ant traps were collected and found with either *A. aegypti* and/or *A. albopictus*, but no *A. seatoi*.

Utilization of bamboo cups by *A. aegypti* (TABLE 1) was more common than anticipated. Incidence varied from 7% to 36% of the *Stegomyia*-positive cups. The highest incidence (36%) occurred in a village-bamboo habitat in Sara Buri Province where the cups were placed within 15 m of houses. The lowest incidence (7%) occurred in a village-orchard habitat in Ang Thong Province during a severe drought. The 28% incidence for *A. aegypti* in the "Hill Top Area" in Sara Buri Province is of interest. This site is a shady grove of old mango trees on a main foot path between villages and approximately 300 to 400 m from the nearest house.

Other Stegomyia

The incidence of *A. annandalei* and *A. gardnerii imitator* found in the surveys was expected, for both commonly utilize natural containers. *Aedes annandalei* exhibits a definite preference to oviposit in bamboo habitats in Thailand, while *A. gardnerii imitator* appears to prefer tree holes, hollow logs and hollow stumps. The absence of *A. annandalei* from the Ang Thong survey cannot be explained, as bamboo was plentiful there.

The extremely low incidence of *A. pseudalbopictus* was surprising. This species is more common in bamboo cup surveys in northern Thailand.

Another species of *Stegomyia* possibly occurs in the areas surveyed, but was not encountered. This species, *A. scutellaris malayensis* Colless, is now known from the following provinces in Thailand: Nonthaburi, Phuket, Prachuap Khiri Khan, Surat Thani and Trat. All these areas are insular or coastal except Nonthaburi, which is just northwest of Bangkok and 45 km from the Gulf of Thailand. This species is typically found in the same larval habitats as *A. albopictus* and currently larval separation of these 2 is very difficult (Huang 1971).

DISCUSSION

These data suggest that *A. seatoi* is a semi-domestic species, with a definite preference for natural container larval sites associated with rural human habitations or activities. Between 1962 and 1970 thousands of *Stegomyia* were collected in Thailand from sylvan and domestic habitats and no *A. seatoi* have been identified from jungle collections or further than 1000 m from human habitations. Natural bamboo containers appear a preferred oviposition site for *A. seatoi*, while oviposition in water jars (ong) appears extremely limited. However, more data from all seasons are needed to clarify *A. seatoi* oviposition preferences.

The *A. aegypti* population found in the abandoned mountain top community in Nakhon Sawan Province is of interest since it had been isolated for over 1 year. Isolation and a reversion to feeding on animals other than man would be prerequisites for the establishment of a feral strain.

Natural container utilization by *A. aegypti* in the rural village habitats deserves serious consideration, for these areas are involved in dengue hemorrhagic fever epidemics like the larger towns and cities of Thailand. The present study, conducted primarily during the dry seasons, revealed *A. aegypti* in 23% (7/30) of the natural containers found with larvae, and up to 100 m from the nearest house, suggesting more than incidental usage. Utilization of natural containers such as bamboo stumps by *A. aegypti* may be much more important during the rainy season, when the number of stumps with water would outnumber the water jars in a village. Macdonald (1956) found an almost identical number (8/31) of containers with *A. aegypti* in rural villages or towns in Malaya; however, no seasonal data were included. Control of *A. aegypti* in villages where natural and artificial containers are utilized could prove difficult and costly. Gould et al. (1970), after insecticide treatment of water jars in a study village, observed a cessation of *A. aegypti* oviposition in bamboo cups previously set in the area, while in a control village *A. aegypti* utilization of set cups continued. They suggested the *A. aegypti* using the bamboo cups were emigrants from the household population. Prior to that control study, surveys had revealed no bamboo stump utilization by *A. aegypti* and low incidence of *A. aegypti* use of natural containers such as tree holes, coconut shells, husks and bracts (Gould et al. 1968).

Natural container utilization by *A. aegypti* in Bangkok has been dismissed as insignificant by Tonn et al. (1969, 1970). However, the area (Soi Aree) where Tonn et al. (1969) reported no natural containers on 302 premises surveyed is the same area where this study reports 4 natural containers with *A. aegypti* larvae and 3 more potential, natural larval sites on just 2 surveyed premises. We contend that further surveys for natural container use by *A. aegypti* are needed in Bangkok like the study conducted in Trinidad by Kellett & Omardeen (1957) and the study initiated in southern India by Rao et al. (1970). Such surveys, particularly in areas where lush vegetation abounds may show significant seasonal utilization of natural containers by *A. aegypti*.

The bamboo cup surveys revealed 22% of the *Stegomyia*-positive cups contained *A. aegypti*, while *A. seatoi* utilized 39% and *A. albopictus* 93%. These data are significant because 57% (27/47) of the *A. aegypti*-positive cups also contained *A. seatoi*, and bamboo cups have thus far been the best method of sampling *A. seatoi* populations. The larvae of these 2 species are so similar that only counting the branches and number of paired hairs in the ventral brush will definitively differentiate them. Consequently, rearing is the most concise way to separate *A. aegypti* from *A. seatoi*, but unfortunately, rearing creates more problems since *A. seatoi* adults are easily confused with adults of *A. albopictus*, *A. pseudalbopictus* and *A. scutellaris malayensis*.

When *A. scutellaris malayensis* is encountered with *A. albopictus* as reported (as *A. scutellaris*) in larval rearing surveys (Yasuno & Tonn 1969), the best way to separate them is by rearing. The reverse is true in areas where *A. albopictus* and *A. pseudalbopictus* are encountered together because their larvae are easily separated, but the adults are only separable on the basis of 1 small character.

Stegomyia surveys in Thailand should be conducted with an increased taxonomic alertness (Huang 1970). Hopefully, *A. seatoi* utilization of water jars will prove insignificant; however, it has been found in this habitat and cannot be ignored. Future *Stegomyia* surveys in small villages should seriously consider natural container breeding by *A. aegypti* and should expect to find *A. aegypti* and *A. seatoi* larvae together, even such associations as *A. aegypti*, *A. albopictus*, *A. annandalei* and *A. seatoi*. Future surveys in Thailand, regardless of procedure, will have to be conducted with extreme taxonomic caution. Those who choose a procedure like the "1 larva per container" technique (Sheppard et al. 1969) should carefully weigh the advantages (speed) against the taxonomic disadvantages (identification).

The knowledge gained from this study is presented primarily to give a basic indication of *A. seatoi* biology and to stimulate interest in further research on this mosquito and other *Stegomyia* species. Further field work is necessary to broaden our knowledge of *A. seatoi*, and will probably reveal a wider distribution, even outside of Thailand. Researchers interested in laboratory biology or disease transmission studies will be pleased to hear that *A. seatoi* is a hardy species, and a colony has been established since June 1970 at SEATO Medical Research Laboratory, Bangkok, Thailand.

Finally, we would like to emphasize that this study was possible only because of recent progress on the taxonomy of the *Stegomyia* of Thailand, and that this progress was possible only through concerted, precise taxonomic studies. Furthermore, the data presented herein demonstrate the value of careful taxonomic studies before undertaking biological, ecological or, when feasible, epidemiological studies.

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